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### Evaluation of the Physiological Quality of Forest Seed Species through the PH Test of the Exudate

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#### ABSTRACT

The pH evaluation of the exudate is a fast test to evaluate the physiological quality of seeds, however, studies with forest species are scarce. Thus, the aim was to verify the possibility to evaluate the physiological quality of forest seed species by the pH test of the exudate using individual and massal methods in different seed imbibition periods. The experimental material consisted of seeds of the species *Aspidosperma parvifolium* (Apocynaceae), *Aspidosperma polyneuron* (Apocynaceae), *Cabralea canjerana* (Meliaceae), *Cariniana legalis* (Lecythidaceae), *Gallsia integrifolia* (Phytolaccaceae), *Handroanthus chrysotrichus* (Bignoniaceae), *Lonchocarpus campestris* (Fabaceae) and *Pterogyne nitens* (Fabaceae). For the individual method, 80 repetitions of a seed were used, which were placed in individual containers containing 50 ml of distilled water. For the massal method, 4 repetitions of 25 seeds were used, which were placed in containers containing 75 mL of distilled water. For both methods the following imbibition periods were evaluated: 2, 4, 6, 8, 24 and 48 hours. The tests were placed in a germinator BOD chamber with a constant temperature of 25 °C. After each imbibition period, the pH reading of the exudate in the phenolphthalein indicator solution in which the seeds were immersed, was taken. The seeds obtained from the individual method were put to germinate in an orderly manner, later to be correlated with germination. The seeds obtained from the massal method were put to germinate randomly. The correlation analysis between pH of the exudate and germination was done. The pH test of the exudate applied by the individual method was more efficient to evaluate the physiological seed quality when compared to the massal method. When efficient, the results obtained in the pH test of the exudate by the massal method corroborate with the results obtained by the individual method, showing to have correlation. The best imbibition periods to evaluate the physiological quality of the seeds were 6 and 8 hours for *Aspidosperma parvifolium* (individual and massal methods); 8 hours for *Aspidosperma polyneuron* and *Cabralea canjerana* (individual method); 48 hours for *Gallsia integrifolia* (individual method); 8 hours for *Handroanthus chrysotrichus* (individual method); 2, 4 (massal method), and 6 hours for *Pterogyne nitens* (individual and massal methods). For the species of *Cabralea canjerana*, *Cariniana legalis* and *Lonchocarpus campestris* the pH tests of the exudate were not efficient due to poor or absent correlation with germination.

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## INTRODUCTION

The seed analysis aims to show the seed quality of a particular lot, these analysis can be done by the means of genetic, physical, physiological and sanitary parameters (Lopes and Nascimento, 2009).

The agility in achieving results becomes indispensable in quality control programs of forest seeds suppliers because they favor decision-making in the supply chain (Fessel *et al.*, 2010).

For native species, which have a major lack of information, quality and vigour analysis aiming to maximize and to make viable their position in the seed market and seedling production are still needed (Sarmento and Villela, 2010).

The pH test of the exudate is based on the integrity of seed membrane systems, has distinguished itself by identifying the deterioration process, and allow measures that can mitigate its effect on seed quality (Menezes, 2013). The test principle is that seeds in advanced degree of deterioration release more H<sup>+</sup> ions, causing the medium in which the seeds are to become more acid (Borghetti and Ferreira, 2004).

The fast seed vigour tests, as the pH test of the exudate obtains information faster, also provides answers that can complement the germination test in a shorter period of time (Gonçalves *et al.*, 2008).

Melo and Martins (2010) and Stallbaun *et al.* (2015) recommend the pH test of the exudate to evaluate the forest seeds quality, because the method is efficient, fast and economic.

Despite the simplicity and quickness of the pH test of the exudate, the evaluation based on the color can lead to an erroneous interpretation. This can occur by the influence of factors such as seed water content, temperature and imbibition time (Santana *et al.*, 1998).

Thus, it is known that the details of the methodology of this test, such as the amount of water to be used and the number of seeds per repetition are unknown for all species but their potential and agility for information about the vigour of a seed lot justify the studies that seek the improvement of the knowledge already obtained (Marcos Filho, 2005).

Therefore, the aim of this study was to verify the possibility to evaluate physiological quality of seeds of different forest species by the individual and massal pH test of the exudate correlating their results with the germination.

## MATERIAL AND METHODS

### **Experimental Material:**

The experiment was conducted at the Seed Analysis Laboratory at the Federal Technological University of Paraná, Campus Dois Vizinhos. The experimental material consisted of the following seed species: *Aspidosperma parvifolium* (Family: Apocynaceae; Common name: guatambu); *Aspidosperma polyneuron* (Family: Apocynaceae; Common name: peroba rosa); *Cabralea canjerana* (Family: Meliaceae; Common name: canjerana); *Cariniana legalis* (Family: Lecythidaceae; Common name: jequitibá); *Gallesia integrifolia* (Family: Phytolaccaceae; Common name: pau d'alho); *Handroanthus chrysotrichus* (Family: Bignoniaceae; Common name: ipê amarelo); *Lonchocarpus campestris* (Family: Fabaceae; Common name: rabo de bugio); *Pterogyne nitens* (Family: Fabaceae; Common name: amendoim do campo).

All seeds were collected in the State of Paraná, in areas of permanent preservation (APP), Legal Reserve (RL), or small forest remnants, and yet, some were provided by the Environmental Institute of Paraná (IAP).

### **pH test of the exudates:**

The pH test of the exudate was done by the individual and massal methods, both evaluating the imbibition periods (treatments) and correlated with germination.

The pH of the solution was indicated using an indicator solution that was prepared based on the recommendations of Cabrera and Peske (2002), which was formulated with 1,0 gram of phenolphthalein dissolved in 100 ml of absolute alcohol and then, added to 100 mL of distilled water.

After the imbibition periods were tested, one drop of the indicator solution was added into each container with the assistance of a micropipette. The reading was done immediately after the contact of the indicator solution with the imbibition solution.

The interpretation of the color of the solution was analysed for the color of the medium. When pink, being considered a viable seed (basic medium), and when transparent indicating that the seed is not viable (acid medium) (Amaral and Peske, 1984).

The germination test according to Carvalho and Nakagawa (2000) is the main criteria used to evaluate the physiological quality of seeds. This should be done under controlled conditions of light, humidity and temperature (Borghetti and Ferreira, 2004).

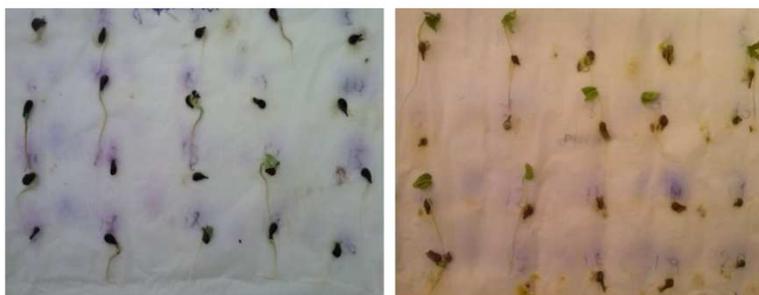
The established conditions for the germination tests were determined from information obtained in the literature for the same species or when not available for the same genre. Martins *et al.* (2011) and Fantinel *et al.* (2013) for *Handroanthus chrysotrichus* (ipê amarelo), Kopper *et al.* (2010) for *Cariniana legalis* (jequitibá), Carvalho *et al.* (2006) for *Aspidosperma polyneuron* (peroba rosa), Silva *et al.* (2007) for *Aspidosperma*

*parvifolium* (guatambu), Santos *et al.* (2008) for *Pterogyne nitens* (amendoim do campo), Barros *et al.* (2005) for *Gallesia integrifolia* (pau d'algo), Lima *et al.* (2008) for *Lonchocarpus campestris* (rabo de bugio), and Grunennvaldt *et al.* (2014) for *Cabralea canjerana* (canjerana).

The predominant condition for germination was under the temperature of 25 °C with a photoperiod of 12 hours of light and 12 hours of dark, using the roll paper substrate, with the exception of the species *Cabralea canjerana* which was wrapped on the substrate on paper in condition of continuous darkness. All substrates were previously sterilized in a vertical autoclave and the germination test were moistened with 2.5 times its weight in distilled water, as determined by the Seed Analysis Rules – RAS. All the tests were allocated in a B.O.D chamber (Biological Demand Oxygen).

The germination was calculated by the formula  $G\% = (N/100) \times 100$ , where: N = number of germinated seeds at the end of the test.

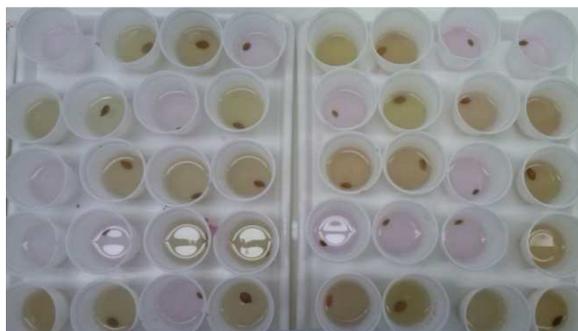
The seeds that attended the botanical criteria germination were considered germinated (Labouriau, 1983; Santos and Aguiar, 2005) (Figure 1).



**Fig. 1:** Germination test on the seeds of *Cariniana legalis* (A) and *Gallesia integrifolia* (B).

#### ***pH of the Individual Exudate:***

For the application of the pH test of the exudate by the individual method, 80 repetitions were used with 1 seed each, which were placed in individual containers containing 50 mL of distilled water (Figure 2) (Amaral and Peske, 1984) and put to soak for 2, 4, 6, 8, 24 and 48 hours (treatments).



**Fig. 2:** pH test of the exudate by the individual method for *Pterogyne nitens* seeds.

At the end of each imbibition period a reading was done with the indicator solution and after the reading the seeds used were put to germinate in an orderly manner, in order to correlate the values (1 for basic medium (pink), 0 for acid medium (colorless) with or without germination of the seedlings. The evaluation compared the color of the exudate of each cell with a control cell with distilled water without seeds (blank test).

#### ***pH of the Massal Exudate:***

For the application of the pH test of the exudate by the massal method, 4 repetitions of 25 seeds were used which were placed in containers containing 75 mL of distilled water (Figure 3) and put to soak for 2, 4, 6, 8, 24 and 48 hours ( treatments ) .

The reading was done in the same way as for the individual method and right after the used seeds were put to germinate for later correlation between pH exudate and germination.



Fig. 3: pH test of the exudate by the massal method for *Pterogyne nitens* seeds.

#### Data Analysis:

The experimental design used was completely randomized in a unifactorial scheme, using 80 repetitions with 1 seed for the individual method and 4 repetitions with 25 seeds for the massal method. The treatments for each method used corresponded to the imbibition periods of the seeds (2, 4, 6, 8, 24 and 48 hours) for the pH reading.

After the compilation, the data set was submitted to the Lilliefors test to verify the normality of the data from the mean and standard deviation, however, there was no need for data transformation. Met the model assumptions, the statistical analysis proceeded.

To measure the degree of association between the variables germination and pH of the exudate aiming to identify the best seed imbibition period, the simple correlation test was applied which is also used by Araldi; Coelho (2015).

The interpretation of the correlation coefficient was given as pointed out by Dancey and Reidy (2006) where:  $r = 0$  indicates no linear relationship between the variables;  $r = 0.10$  to  $0.30$  corresponds to a weak correlation;  $r = 0.40$  to  $0.60$  moderate correlation;  $r = 0.70$  to  $1$  strong correlation (for both cases, negative or positive). The tests applied were considered efficient when the correlation between pH and germination was classified as strong.

## RESULTS AND DISCUSSION

What occurs when a seed is not viable or has low vigour is the release of exudates, causing a pH increase. The data tabulation has established the value 1 for solutions with a pink color (basic medium), zero for solutions that showed no change in color (acid medium), 1 to germinated seeds and zero for non-germinated seeds. Thus, the correlation that should be observed to confirm the relation between the variables needed to be strong and positive.

According to Cabrera and Peske (2002), Carvalho *et al.* (2002), Peske and Amaral (1986) and Rech *et al.* (1999), during imbibition the release of organic acids, sugars and ions favor for the solution in which the seeds are to be acid and consequently there is a decrease in the pH of the seed exudates.

Given the above, it can be stated that seeds with greater damage have higher leaching and exudates with greater buffer capacity, which results in a change of the medium coloration in which the seeds are making it colorless, resulting in low pH values (Araldi and Coelho, 2015).

#### pH of the Individual Exudate:

Table 1 shows the correlation coefficients between pH and germination by the individual method in different periods of seed imbibition.

Table 1: Correlation coefficients ( $r$ ) between pH of the exudate and germination by the individual method in different periods of seed imbibition.

TIME	2 HOURS	4 HOURS	6 HOURS	8 HOURS	24 HOURS	48 HOURS
SPECIES						
<i>A. parvifolium</i>	0	0,0657	0,6244	0,8290	0	0
<i>A. polyneuron</i>	0	0	0	0,7580	0,1125	0
<i>C. canjerana</i>	0	0	0,1196	0,8401	0	0
<i>C. legalis</i>	0	0	0	0	0	0
<i>G. integrifolia</i>	0	0	0	0	-0,2075	0,3396
<i>H. chrysotrichus</i>	0	0	0	1	0	0
<i>L. campestris</i>	0	0	0	0	0	0
<i>P. nitens</i>	0,1972	0,2518	0,9497	0,4025	0,3985	0,2523

For *Aspidosperma parvifolium* the best time to evaluate the physiological quality of seeds by the individual method was 8 hours and then 6 hours, both showing a strong correlation. Also for *Aspidosperma polyneuron* and *Cabrera canjerana* seeds the best imbibition period was 8 hours, showing a strong correlation.

For *Cariniana legalis* and *Lonchocarpus campestris* the correlation was zero, which shows that normal seedlings and non-germinated seeds showed the same pH coloration of the medium they were soaked.

The best imbibition period for *Gallesia integrifolia* was 48 hours, however, there was a weak correlation between the pH of the medium and germination.

*Handroanthus chrysotrichus* seeds showed better correlation between pH and germination within 8 hours of imbibition, with a strong and perfect correlation.

For the specie *Pterogyne nitens* at 6 hours of imbibition a strong correlation was observed, the color pink was observed for germinated seeds and a colorless medium for non-germinated seeds.

Melo and Martins (2010) evaluating the physiological quality of seeds through the pH test by the individual method, recommended the imbibition for 90 minutes for *Tabebuia serratifolia* and 30 minutes for *Tabebuia ochracea* (synonym *Handroanthus*). These results are contradictory to the ones found in the present study with *Handroanthus chrysotrichus* seeds, which needed an imbibition period considerably higher for later evaluation.

Stallbaun *et al.* (2015) evaluating the physiological quality of *Anadenanthera falcata* (Angico – Fabaceae) using the pH test of the exudate by individual method, observed that it was more efficient and judicious for vigour analysis of the seeds when compared to the massal method.

For *Annona squamosa* (Pinha – Annonaceae), Araújo *et al.* (2014) recommended the pH test of the exudate by the individual method, however, there was no difference between the imbibition periods (30, 60 and 90 minutes). Araldi and Coelho (2015) recommended the pH test of the exudate at during the period of 30 minutes for *Araucaria angustifolia* seeds.

### **pH of the Massal Exudate:**

Table 2 shows the correlation coefficients between pH and germination by the massal method in different periods of seed imbibition.

**Table 2:** Correlation coefficients (r) between pH of the exudate and germination by the massal method in different periods of seed imbibition.

TIME SPECIES	2 HOURS	4 HOURS	6 HOURS	8 HOURS	24 HOURS	48 HOURS
<i>A. parvifolium</i>	0	0	0,3015	0,7071	0	0
<i>A. polyneuron</i>	0	0	0	-0,9899	0	0
<i>C. canjerana</i>	0	0	0	-1	0,4082	0
<i>C. legalis</i>	0	0	0	0	0	0
<i>G. integrifolia</i>	0	0	0	0	0	0
<i>H. chrysotrichus</i>	0	0	0	0	0	0
<i>L. campestris</i>	0	0	0	0,5773	0	0
<i>P. nitens</i>	0,8628	0,9428	0,8432	0	0	0

For *Aspidosperma parvifolium* the best time to evaluate the physiological quality of seeds by the massal method was 8 hours, which was also observed in the individual method. For the *Aspidosperma polyneuron* seeds no period was efficient to evaluate seed quality by the massal method.

With seeds of *Cabralea canjerana* there was only a moderate tending to weak correlation in a 24 hour period. However, for *Cariniana legalis*, *Gallesia integrifolia*, and *Handroanthus chrysotrichus* the correlation was null. Normal seedlings and non-germinated seeds showed the same pH coloring of the medium and the massal method was not efficient to evaluate the physiological quality of seeds of these species within the analyzed periods.

The best imbibition period for *Lonchocarpus campestris* was 8 hours, being a moderate correlation. For *Pterogyne nitens* species in the periods of 2, 4 and 6 hours of imbibition there was a strong correlation.

About the treatments that did not present any degree of correlation, it was observed that all the samples showed a single color and different germination percentage. It was then found that the pH test of the exudate by the massal method was efficient only to evaluate the seed quality of two tested species, *Aspidosperma parvifolium* and *Pterogyne nitens*. The individual method was efficient for the following species: *Aspidosperma parvifolium*, *Aspidosperma polyneuron*, *Cabralea canjerana*, *Handroanthus chrysotrichus*, and *Pterogyne nitens*. When the massal method was efficient the results corroborate to those found in the individual method for imbibition period more suitable to evaluate the quality of seeds.

Matos (2009) evaluating the physiological quality of *Anadenanthera falcata* (Angico – Fabaceae) seeds, recommended imbibition period of 30 minutes pointing a germination viability of 82.5 %. This result does not corroborate to the one found for *Lonchocarpus campestris* and *Pterogyne nites* seeds which belong to the same family and needed longer periods of imbibition to evaluate the quality of seeds by the massal method.

Gomes (2013), evaluating the physiological quality of *Terminalia argentea* seeds (Capitão do mato – Combretaceae) by the pH test of the exudate using the massal method, found that the imbibition period of 30 minutes was efficient in the separation of seed lots.

According to Hilst (2009), the pH test of the exudate was promising to evaluate the viability of *Coffea arabica* L. seeds (Café - Rubiaceae), however, only after a imbibition period of 72, 96 and 120 hours.

For the seeds of *Guazuma ulmifolia* (Mutambo – Malvaceae) the pH test of the exudate by the massal method did not estimate the viability of seeds, because there was no relationship of color to the emergency values (Barboza *et al.*, 2014).

Santos *et al.* (2015) studied the pH test of the exudate in *Dalbergia miscolobium* seeds (Angico – Fabaceae) and concluded that phenolphthalein solutions do not present compatible results with the results obtained by the pH meter. This result suggests using other indicator solutions, such as, for example, Yamada and bromothymol blue used by the same authors, which are efficient and corroborate the results obtained by the pH meter.

According to Matos (2014) colorimetric method is not efficient when using phenolphthalein solution proposed by Cabrera and Peske (2002), they are not able to differentiate small changes in the leach of H<sup>+</sup>, being necessary to evaluate other indicator solutions. Stallbaun *et al.* (2015) also observed an overestimation of the results in the massal method when compared to the individual method.

When used in this study the solution proposed by Cabrera and Peske (2002) also overestimated the results obtained, so that the pink color was observed in most cases also for germinated seeds.

For corn seeds the authors Cabrera and Peske (2002) recommended the pH test of the exudate through the massal method, which showed high reliability. However, they said that the test can not distinguish a sample containing many high-quality seeds and a few dead seeds of another sample with all good quality seeds.

Thus, it is important to emphasize that for forest seeds this event may be aggravated due to poor homogeneity of seeds compared to seeds of cultivated species, and that the vigor tests when applied in greater heterogeneity seeds are going to evaluate better the performance of the lots at the field level (Spina and Carvalho, 1986). Before the facts it is clear that more detailed studies should be conducted to improve this test application technique for both methods and species studied.

It can often be noted that the acid reading in the imbibition medium does not impair germination. Santos *et al.* (2015) found that the seeds of *Dalbergia miscolobium* that present acid exudates germinate on an average of 80%. This fact can prove that the acidification of the imbibition solution is not sufficient to conclude that the seed is not viable or the indicator solution is not efficient.

Another factor observed was that seeds of some species can release exudates with sharper coloring, which ultimately affects reading through the colorimetric method. This event could be seen for *Pterogyne nitens* and it was also described by Matos (2014) with seeds of *Dalbergia miscolobium*. Also more pronounced coloring was observed through the release of exudates from the seeds of *Aspidosperma parvifolium*, *Aspidosperma polyneuron*, *Cabralea canjerana*, and *Lonchocarpus campestris*, however, not culminating in low viability thereof.

Having the results of correlation between pH and germination it is possible to check the reliability or not of the pH test of the exudate. Imbibition periods of the seeds, water availability, other indicator solutions among others, which enable greater understanding and clarification of the best technique to be applied. These should be studied in greater depth, to improve the accuracy of the pH test of the exudate by the individual and massal methods, to estimate the viability of forest seeds of the studied species.

### Conclusions:

The pH test of the exudate applied by individual method was more efficient and judicious to evaluate the physiological quality of seeds of the studied specie, when compared to the massal method.

When efficient, the results obtained in the pH test of the exudate by the massal method corroborate the results obtained by the individual method, showing correlation.

However, upon the conditions in which they were conducted, the following conclusions can be done as to the best imbibition periods to evaluate the physiological quality of seeds:

- 6 and 8 hours for *Aspidosperma parvifolium* (individual and massal methods);
- 8 hours for *Aspidosperma polyneuron* e *Cabralea canjerana* (individual method);
- 48 hours for *Gallesia integrifolia* (individual method);
- 8 hours for *Handroanthus chrysotrichus* (individual method);
- 2, 4 (massal method), and 6 hours for *Pterogyne nitens* (individual and massal methods).

For the species of *Cabralea canjerana*, *Cariniana legalis* and *Lonchocarpus campestris* the pH tests of the exudate in both methods were not efficient due to weak or nonexistent correlation between germination and pH.

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